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## Molecular modelling study for chiral separation of equol enantiomers by $\beta$ -cyclodextrin

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## Abstract

The intermolecular forces responsible for complexation of equol, a chiral molecule, with  $\beta$ -cyclodextrin are determined using a molecular modelling study. The differential interactions between each enantiomer and the chiral host give rise to different configurations for the corresponding inclusion complexes which give rise to enantiodifferentiation. The van der Waals term is the main contributor to the total potential; however, the electrostatic term influences the enantioselectivity significantly since it establishes a difference between the most stable position of R- and S-equol and hence between their energies. A statistical analysis of the minimized energies is carried out to determine that R-equol is more retained than S-equol.

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## 1. Introduction

Compounds that exist in two forms that are nonsuperimposable mirror images (enantiomers) are optically active, rotating plane-polarised light in opposite directions. This property is shown by an asymmetric carbon atom (i.e., one with four different substituents) and also by other atoms. Many compounds, such as drugs, agrochemicals and food additives have been marketed as racemic mixtures (approximately equal proportions of enantiomers), but the often dramatic difference in biological effects of the enantiomers of a chiral compound demonstrates the need for methods capable of discriminating between them. During the last decade, advances in the science and technology of enantiomer resolution have been made. Presently, the use of high-performance liquid chromatography (HPLC) is the most common means of achieving enantioselective separation [1]. A large number of different chiral stationary phases (CSPs) are available classified according to separation mechanisms and thus the structural requirements of the analyte [2]. Chiral cavity type phases such as cyclodextrins (CD) and their derivatives are in widespread use. CDs are macrocyclic molecules composed of glucose units (7 for  $\beta$ -CD) forming truncated cone-shaped compounds and their ability for chiral recognition is mainly due to the formation of inclusion complexes [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13].

Equol is an 'oestrogen-like' isoflavonoid compound and its nuclear magnetic resonance spectrum is identical to that of 3,4-dihydro-3-(4-hydroxyphenyl)-2*H*-1-benzopyran-7-ol (Fig. 1). It is a potent inhibitor of the Na-K-Cl cotransport system and is purified from salt-loaded rat urine [14]. Equol possesses an asymmetric carbon, i.e. it is a chiral compound, although so far it is not clear which enantiomer occurs in nature.

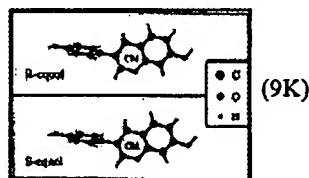


Fig. 1. Structure and composition of equol molecules. CM is their centre of mass.

The aim of theoretical studies is to discover why and how the chiral recognition takes place, taking into account that the nature of the forces responsible for one enantiomer binding to a chiral surface is the same as that for its optical isomer. There are no rules about what molecular modelling method is to be applied, but some factors influencing the choice are: molecular size and shape of analyte and CSP, data available, physico-chemical properties, etc. [2]. In this study we use a model based on molecular mechanics, previously applied to examine the formation of inclusion complexes of  $\beta$ -CD with another guest molecules [11], obtaining results in agreement with experiment.

To study the enantiodifferentiation of R- and S-equol by  $\beta$ -CD we first determine the interaction energy between the chiral host and each enantiomer, to establish the differences in the corresponding inclusion complexes. Section 2 is devoted to the presentation of molecules and to the interaction potential between  $\beta$ -CD and the enantiomers. Differences in the potential energy and inclusion complex geometries for both isomers, along with the retention order, are discussed in Section 3.

## 2. The model

The atomic coordinates of  $\beta$ -CD were taken from the literature [15] and the geometries of both enantiomers of equol were calculated using the AM1 semi-empirical Hamiltonian [16] included in the MOPAC 6.0 package [17]. In all cases, the presence of minima was confirmed by inspection of the Hessian.

The lowest-energy configuration of host-guest complexes formed by CD with both enantiomers was determined assuming inclusion complex formation does not affect the structure of either molecule, keeping the internal coordinates of both guest and host fixed. In this work the molecules are described by the all-atom model as this offers a somewhat better description of the potential surface. The intermolecular energy  $E$  is represented by contributions from van der Waals, hydrogen-bonding and electrostatic functions, as in the AMBER force field [18, 19].

$$E = \sum_{i,j} \left[ \frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^6} + \frac{q_i q_j}{R_{ij}} \right] + \sum_{\text{H-bonds}} \left[ \frac{C_{ij}}{R_{ij}^{13}} - \frac{D_{ij}}{R_{ij}^{10}} \right], \quad (1)$$

where  $R_{ij}$  represents the distance between the  $i$ th atom of the guest molecule and the  $j$ th atom of  $\beta$ -CD. A 6-12 function is used to simulate the van der Waals interactions. The attractive London dispersion interaction between two atoms is described by a  $r^{-6}$  term and the repulsive interaction caused by Pauli exclusion is given by a  $r^{-12}$  term. The van der Waals function is replaced by a 10-12 function for those pairs of atoms which can participate in H-bonding [18, 19, 20, 21]. A hydrogen bond is deemed present when the O-O distance is  $<2.5$  Å and if the O-H...O angle is  $>120^\circ$  [7]. The electrostatic interaction is calculated by a Coulomb-type potential. The corresponding parameters employed ( $A_{ij}$ ,  $B_{ij}$ ,  $C_{ij}$  and  $D_{ij}$ ) and the atomic point charges are the AMBER force field parameters [18, 22].

The Boltzmann average energy is evaluated at 300 K.

## 3. Numerical results and discussion

### 3.1. Numerical background

A Cartesian frame was chosen with the Z axis collinear with the axis of the CD ring (thus the XY plane is parallel to the faces of the CD), and the origin of the coordinates lies approximately at the centre of the cavity. The position of the guest molecule is defined by the coordinates  $(x_0, y_0, z_0)$  of its centre of mass, and its orientation by its Euler angles with respect to the absolute frame.

For each position  $(x_0, y_0, z_0)$  of the guest molecule, the potential energy is calculated for the different orientations of the molecule which result in rotation of its Euler angles, and the minimum of these values is assigned to the position  $(x_0, y_0, z_0)$  [11, 12, 13]. The path for each axis ( $X, Y, Z$ ) is 0.1 Å and for the Euler angles  $30^\circ$ .

The position and orientation of the guest molecule for which we obtain the lowest potential energy gives the geometry of the inclusion complex for both enantiomers. The curve joining the minimum energy for every plane  $Z=\text{constant}$ , characterizes the penetration potential, and describes the variation of  $E$  ( $E_R$  and  $E_S$  for R- and S-enantiomer, respectively) along the Z axis. These results establish: (a) the intermolecular forces responsible for analyte binding to the CSP; (b) the differential interactions between each enantiomer and the chiral host, giving rise to different configurations for both; and (c) the more retained enantiomer of equol, corresponding to the more stable structure.

We can make the assumption that computational errors in the complexes of one enantiomeric form will cancel errors in the other, thus producing meaningful small energy differences.

### 3.2. Binding energies

Fig. 2 shows the penetration potential for R- and S-equol. The origin of the Z axis is at the centre of the cavity and the narrow rim of the CD corresponds to the negative Z axis. It presents some aspects already observed in the interaction of  $\beta$ -CD with other guest molecules [4, 5, 6, 7, 8, 9, 10, 11] and some new characteristics:

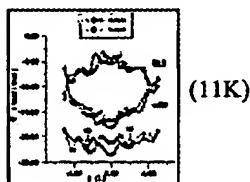


Fig. 2. The van der Waals term (vdW), electrostatic contribution (ELE) and total energy for the interaction between  $\beta$ -CD and each enantiomer. The relative minima marked by arrows correspond to the configurations (b) (Fig. 3) for R-equol and (d) (Fig. 4) for S-equol.

(a) There is no H-bonding inside the cavity, this term only contributes to the total energy when the centre of mass of the guest molecule is outside the cavity, near the faces of the CD. Therefore, this term does not affect the formation of the inclusion complex because its

greatest contribution to the total energy is ~2.7% and corresponds to the positions given in Table 1 (Figs. 3a and 4a).

Table 1. Total energy and contributions of van der Waals, electrostatic and H-bonding terms in kcal mol<sup>-1</sup>

	<i>E</i>	vdW	ELE	H-bonding	<i>X</i>	<i>Y</i>	<i>Z</i>
R-equol	-18.981	-12.387	-6.128	-0.465	-2.5	-2.0	-4.0
S-equol	-18.485	-11.386	-6.600	-0.498	0.5	1.0	-4.0
R-equol	-24.645	-18.454	-6.190	0.0	-0.6	0.0	-0.2
S-equol	-23.796	-15.356	-8.438	0.0	0.6	-1.4	-2.3

#### Boltzmann averages:

R-equol	-21.701
S-equol	-21.191

The position of the enantiomer centre of mass is expressed in Å and the orientation of the molecules is represented in Figs. 3a, 4a, 3d and 4b, respectively.

(b) The van der Waals term is the main contribution to the total energy, both inside and outside the cavity, and then it determines the configuration of the guest molecule directly. The behaviour of this term resembles the shape of a well potential since its minimum is located at the centre of the cavity and increases near the ends of the CD.

(c) The electrostatic term, on the contrary, is smaller (more stabilized) outside the cavity and increases inwards toward the cavity centre almost resembling a potential barrier. The main feature of this term is its shape in the region of the cavity centre where it presents two peaks separated by a small well. The highest value of this contribution is about -3.4 kcal mol<sup>-1</sup> and occurs when the centre of mass of R-equol is approximately on the plane determined by the C6 primary hydroxyl group of the glucose units and on the plane determined by the C5 atoms (linked to the C6 primary hydroxyl group [3, 8]) for the S analyte. The secondary maximum corresponds approximately to the guest molecule centre of mass on the plane determined by the atoms C2 and C3 of the β-CD ring. Inside the cavity this term can contribute 20–50% to the total energy and modifies the shape of the van der Waals potential, mainly from the narrow rim to the middle of the cavity.

### 3.3. Inclusion complexes

Fig. 3 and Fig. 4 exhibit the evolution of R- and S-equol throughout the cavity to form inclusion complexes (Figs. 3d and 4b). Note that the front atoms of the CD cavity have been left aside for clarity. The centre of mass of the guest molecule is placed near the narrow rim, approximately on the plane determined by the C6 primary hydroxyl group, for S-equol and at the middle of the cavity for R-equol. It can be seen that the evolution of the enantiomers near the ends of the CD is similar, they tend to locate themselves on the cavity axis and to introduce the double ring inside the CD. However, the transition between Fig. 3c and d for R-equol (Fig. 4b and c for S-equol) involves a 180° change in the guest molecule orientation, but in this case the guest cannot revolve in the XY plane inside the cavity due to its size. Therefore, according to the face from which the enantiomer approaches the CD it may

achieve a stable position (Figs. 3b and 4d) which does not correspond to the lowest energy. The relative minima corresponding to these configurations are marked by arrows in Fig. 2. This behaviour is also observed in other studies [11, 12, 13] and its influence on enantioselectivity can be taken into account through a statistical analysis of the calculated energies.

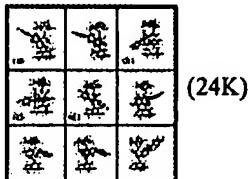


Fig. 3. Evolution of R-equol throughout the  $\beta$ -CD. The coloured atoms belong to the guest molecule. Note that the front atoms of the CD cavity have been left out for clarity. (a) Position of R-equol corresponding to the largest contribution of the H-bonding term to the total energy. (b) Position of R-equol corresponding to the relative minimum marked by the arrow in Fig. 2. (d) Inclusion complex.

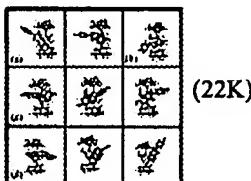


Fig. 4. Evolution of S-equol throughout the  $\beta$ -CD. The coloured atoms belong to the guest molecule. Note that the front atoms of the CD cavity have been left out for clarity. (a) Position of S-equol corresponding to the largest contribution of the H-bonding term to the total energy. (b) Inclusion complex. (d) Position of S-equol corresponding to the relative minimum marked by the arrow in Fig. 2.

### 3.4. Chiral recognition

On comparing the total energy for R- and S-equol (Fig. 2) one appreciates there are no meaningful differences outside the cavity. Between the different contributions, the H-bonding term practically does not influence the enantiodiscrimination due to its small contribution to the total potential.

Inside the cavity the main differences between  $E_R$  and  $E_S$  are observed from the narrow rim to the middle of the cavity and they are due to the Lennard-Jones potential, closely connected (through the parameters  $A_{ij}$ ,  $B_{ij}$ ) to the spatial distribution of the atoms in the two systems, host and guest. The differences in this term are increased by the electrostatic contribution which, although it shows a similar shape for both enantiomers, is displaced along the Z axis. This makes the minima of the total energy and of the van der Waals potential disagree for S-equol, and the most stable position for this enantiomer is not located at the cavity centre, as happens for R-equol. Therefore, in this case, the Coulombic forces contribute significantly to

the enantiodifferentiation; while the van der Waals term is the main contributor to  $E$  and therefore determines the orientation of the guest molecule, it is the electrostatic term that causes the difference between the most stable position of R- and S-equol and thus between their energies.

The values of the lowest energy, the different contributions and the position of the centre of mass for the enantiomers are given in Table 1. The most stable inclusion complex is formed by R-equol as its total energy is lower than that of S-equol and the difference is  $\sim 0.85 \text{ kcal mol}^{-1}$ . However, there are numerous studies of enantiodiscrimination based on MM concluding that, in general, the chromatographic retention order predicted by the lowest computed energies using rigid molecules, are unable to reproduce experimental results [4, 5, 6, 7, 8, 9]. Therefore a statistical analysis of the minimized energies or a further full relaxation of the complexes is necessary to obtain an elution order in agreement with experimental observation. In this case the free energy difference at 300 K also predict the R enantiomer will be bound tighter, by  $0.53 \text{ kcal mol}^{-1}$ . The entropies of the formation of the inclusion complexes with each enantiomer are  $6.908$  and  $6.844 \text{ cal mol}^{-1} \text{ K}^{-1}$  for R- and S-equol, respectively, and then they do not contribute significantly to the enantiodifferentiation since the enthalpy difference is  $0.51 \text{ kcal mol}^{-1}$  (Table 1). However, the MM calculations in vacuo yield in general isoentropic behaviour and this obviously contradicts the experimental findings, pointing to the importance of solvation effects to entropic contributions in real systems [23]. The importance of solvation effects and structural relaxation of molecules ( $\beta$ -CD and equol) will be the subject of a further paper. The computed energies described in this study are the starting points for this work.

## 4. Conclusions

In this work we attempt to determine the intermolecular forces responsible for complexation of equol with  $\beta$ -CD and the mechanism of enantiodifferentiation. Using a molecular modelling study we found that the largest contribution of the H-bonding term to the total energy is  $\sim 2.7\%$ , when the centre of mass of the guest molecule is outside the cavity near the faces of the CD. The van der Waals term is the main contributor to the total energy and its minimum is located at the centre of the CD ring for each enantiomer. Inside the cavity, the electrostatic term can contribute 20–50% to the total energy and it influences the enantiodifferentiation significantly as it establishes the difference between the most stable position of R- and S-equol and thus between their energies. For S-equol the lowest energy corresponds to its centre of mass placed approximately on the plane determined by the C6 primary hydroxyl group, while for  $E_R$  it is located at the cavity centre. The minimized energy and the free energy for R-equol are lower than those of S-equol, by  $\sim 0.53 \text{ kcal mol}^{-1}$ .

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